

Identification and Antimicrobial Susceptibility of *Alcaligenes xylosoxidans* Isolated from Patients with Cystic Fibrosis

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In the past decade, potential pathogens, including *Alcaligenes* species, have been increasingly recovered from cystic fibrosis (CF) patients. Accurate identification of multiply antibiotic-resistant gram-negative bacilli is critical to understanding the epidemiology and clinical implications of emerging pathogens in CF. We examined the frequency of correct identification of *Alcaligenes* spp. by microbiology laboratories affiliated with American CF patient care centers. Selective media, an exotoxin A probe for *Pseudomonas aeruginosa*, and a commercial identification assay, API 20 NE, were used for identification. The activity of antimicrobial agents against these clinical isolates was determined. A total of 106 strains from 78 patients from 49 CF centers in 22 states were studied. Most (89%) were correctly identified by the referring laboratories as *Alcaligenes xylosoxidans*. However, 12 (11%) strains were misidentified; these were found to be *P. aeruginosa* ($n = 10$), *Stenotrophomonas maltophilia* ($n = 1$), and *Burkholderia cepacia* ($n = 1$). Minocycline, imipenem, meropenem, piperacillin, and piperacillin-tazobactam were the most active since 51, 59, 51, 50, and 55% of strains, respectively, were inhibited. High concentrations of colistin (100 and 200 $\mu\text{g/ml}$) inhibited 92% of strains. Chloramphenicol paired with minocycline and ciprofloxacin paired with either imipenem or meropenem were the most active combinations and inhibited 40 and 32%, respectively, of strains. Selective media and biochemical identification proved to be useful strategies for distinguishing *A. xylosoxidans* from other CF pathogens. Standards for processing CF specimens should be developed, and the optimal method for antimicrobial susceptibility testing of *A. xylosoxidans* should be determined.

Cystic fibrosis (CF) is the most common life-shortening disease caused by a recessive autosomal gene in the Caucasian population and afflicts about 60,000 patients worldwide, approximately 30,000 of whom are cared for in the United States (21). While the genetic defect in CF is known, the relationship between abnormal CF transmembrane conductance regulator and chronic pulmonary disease is not yet fully understood (22). Chronic pulmonary disease is characterized by a vicious cycle of inflammation and infection and is the major cause of morbidity and mortality. The pathogens of CF are well characterized and include *Staphylococcus aureus*, nontypeable *Haemophilus influenzae*, *Pseudomonas aeruginosa*, and *Burkholderia cepacia* (10).

During the past decade, several additional potential pathogens have been recovered from CF patients, including nontuberculous mycobacteria (19), *Stenotrophomonas maltophilia* (3), and *Alcaligenes* species, particularly *Alcaligenes xylosoxidans* (29). As reported to the U.S. Cystic Fibrosis Foundation's National Patient Registry in 1996 and 1997, 1.9 and 2.7% of all CF patients harbored *Alcaligenes* spp. (7, 8). As determined by a research laboratory participating in a phase III trial of aerosolized tobramycin in CF patients who were ≥ 6 years of age, 8.7% of 520 patients harbored *A. xylosoxidans* (1). At present, it is unclear if *A. xylosoxidans* causes clinical disease in CF patients or merely colonizes the respiratory tract (4, 17).

However, accurate identification of multiply antibiotic-resistant gram-negative bacilli isolated from CF patients is critical to facilitate our understanding of the epidemiology of emerging pathogens. Furthermore, accurate identification has important implications for treatment and for the institution of infection control standards if patient-to-patient transmission is observed (26). We examined the frequency of misidentification of *Alcaligenes* spp. by clinical microbiology laboratories affiliated with CF care centers in the United States and determined the antimicrobial susceptibility of these clinical isolates using the National Committee for Clinical Laboratory Standards (NCCLS) interpretative criteria for *P. aeruginosa*, since breakpoints for *Alcaligenes* spp. have not been established.

MATERIALS AND METHODS

Isolates tested. Clinical isolates sent to the Cystic Fibrosis Referral Center at Columbia University (24) from 1995 to 1998 identified by the referring clinical microbiology laboratories as *A. xylosoxidans*, *Alcaligenes* spp., or *Alcaligenes odorans* were selected for this study. All strains were isolated from CF patients cared for at accredited care centers in the United States (6). Strains had been stored at -70°C and were grown on biplates of blood agar and MacConkey agar (REMEL, Lenexa, Kans.) to confirm purity.

Several control strains were used to confirm the identification strategies used: *P. aeruginosa* ATCC 27853, clinical isolates of *P. aeruginosa* (one mucoid and one nonmucoid), *A. xylosoxidans* ATCC 27061 and ATCC 35655, *B. cepacia* ATCC 25416, a clinical strain of *B. cepacia*, *S. maltophilia* ATCC 13637, and a clinical strain of *S. maltophilia*. All clinical strains were isolated from CF patients.

Identification strategy for *Alcaligenes* spp. Isolates were confirmed as *A. xylosoxidans* or *Alcaligenes* spp. using phenotypic and genetic characteristics to distinguish *Alcaligenes* spp. from *B. cepacia* complex, *S. maltophilia*, or *P. aeruginosa*. Isolates were plated on several agar media that included DNase, namely, oxidative-fermentative polymyxin B-bacitracin-lactose (OFPBL) and Mueller-Hinton agar (BBL, Sparks, Md.), and were probed for the exotoxin A gene of *P. aeruginosa* (25). Plates were incubated at 35°C for 18 to 24 h and examined for

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TABLE 1. Identification of 106 strains previously identified as *A. xylosoxidans*, *Alcaligenes* spp., or *A. odorans* from CF patients

Species identification by API zone	No. of strains	<i>exotoxin A</i> probe hybridization	Growth on OFPBL	Production of DNase	Species identification in this study
<i>A. xylosoxidans</i>	94	Negative	No	No	<i>A. xylosoxidans</i>
<i>P. aeruginosa</i>	9	Positive	No	No	<i>P. aeruginosa</i>
<i>Pseudomonas</i> spp.	1	Positive	No	No	<i>P. aeruginosa</i>
<i>B. cepacia</i>	1	Negative	Yes	No	<i>B. cepacia</i>
<i>S. maltophilia</i>	1	Negative	No	Yes	<i>S. maltophilia</i>

growth, pigment production consistent with that of *P. aeruginosa*, and DNase production consistent with that of *S. maltophilia*. In addition, API 20 NE was used to identify the isolates to the species level (bioMérieux, Vitek, Hazelwood, Mo.).

Antimicrobial susceptibility and synergy testing. Antimicrobial susceptibility testing was performed by microbroth dilution assay using commercially prepared microtiter plates (Microtech Medical Systems, Inc., Aurora, Colo.) containing serial twofold dilutions of antibiotics (24). This assay has been shown to be comparable to agar dilution for testing the antimicrobial susceptibility of *P. aeruginosa* isolates from CF patients (25). As there are not yet NCCLS interpretative criteria for susceptibility breakpoints for *Alcaligenes* spp., the breakpoints for *P. aeruginosa* were used. The antimicrobial agents tested included ticarcillin-clavulanic acid (4 to 128 µg/ml), piperacillin (4 to 128 µg/ml), piperacillin-tazobactam (piperacillin component, 4 to 128 µg/ml), ceftazidime (1 to 64 µg/ml), imipenem (0.5 to 16 µg/ml), meropenem (0.5 to 16 µg/ml), ciprofloxacin (0.25 to 8 µg/ml), tobramycin (0.5 to 256 µg/ml), trimethoprim-sulfamethoxazole (trimethoprim component, 0.5 to 16 µg/ml), chloramphenicol (1 to 64 µg/ml), and minocycline (1 to 32 µg/ml). In addition to standard concentrations of antibiotics, the activity of higher concentrations of tobramycin (MIC, 16 to 256 µg/ml) such as those which could be achieved by aerosolization (20) and high concentrations of colistin (100 and 200 µg/ml) were tested. Plates were incubated at 35°C for 18 to 24 h.

Synergy studies using checkerboard dilutions of pairs of antimicrobial agents tested at clinically achievable concentrations were performed (24). The pairs of agents selected had different mechanisms of action and had been shown to have activity against *B. cepacia* complex (2). Fractional inhibitory concentrations (FIC) were calculated as previously described (24). FIC of <0.5 were considered synergistic, and FIC of 0.5 to <1.0 were considered additive.

RESULTS

Identification of isolates. A total of 106 strains from 78 patients (one to five strains per patient) from 49 CF centers in 22 states were tested in this study. The vast majority (99 of 106; 93%) were identified as *A. xylosoxidans* by the referring microbiology laboratories, while six were labeled *Alcaligenes* spp. and one was labeled *Alcaligenes odorans*. Of these 106 strains, 94 (89%) did not grow on OFPBL medium, did not express DNase, did not hybridize with the *exotoxin A* probe, and were identified by API 20 NE as *A. xylosoxidans* as shown in Table 1.

However, 12 strains exhibited phenotypic and/or genotypic properties that were not consistent with those of *Alcaligenes* spp. (Table 1). One strain expressed DNase, did not hybridize with the *exotoxin A* probe, and was identified by API 20 NE as *S. maltophilia*. One strain grew on OFPBL medium, was identified as *B. cepacia* by API 20 NE, and was confirmed as such by the *B. cepacia* reference laboratory (16). Ten strains hybridized with the probe for *exotoxin A*, including four pigment-producing strains. API 20 NE identified 9 of these 10 strains as *P. aeruginosa* and 1 as a *Pseudomonas* sp.

Antimicrobial susceptibility and synergy testing. The 94 *A. xylosoxidans* strains were highly resistant to antibiotics. Minocycline, imipenem, meropenem, piperacillin, and piperacillin-tazobactam had the most activity and inhibited 51, 59, 51, 50,

and 55% of strains, respectively (Table 2). However, the majority of strains were resistant to the remaining agents tested.

We also examined the activity of higher concentrations of tobramycin and colistin. The majority of strains (97%) were resistant to conventional concentrations of tobramycin (MIC, >8 µg/ml). However, 38 (41%) strains were inhibited by concentrations of tobramycin achievable by aerosolization (MIC, 16 to 256 µg/ml), while the MICs for 53 (56%) strains were >256 µg/ml. Only 8% of the *A. xylosoxidans* strains studied were resistant to the high concentrations of colistin tested.

Combinations of antimicrobial agents had some synergistic activity, but the FIC was <0.5 for fewer than 10% of the isolates tested (Table 3). However, additive activity was noted for some combinations, including chloramphenicol-minocycline (35%), ciprofloxacin-imipenem (27%), and ciprofloxacin-meropenem (23%).

DISCUSSION

Most (89%) isolates from CF patients identified as *A. xylosoxidans* or *Alcaligenes* spp. by referring clinical microbiology laboratories affiliated with U.S. CF care centers were correctly identified. However, three other gram-negative bacilli were incorrectly identified as *A. xylosoxidans*, as 12 (11%) strains were identified as other CF pathogens, including 10 as *P. aeruginosa*, 1 as *S. maltophilia*, and 1 as *B. cepacia*. Used together, selective media, biochemical identification, and molecular detection of *exotoxin A* proved to be useful strategies. In this study, API 20 NE consistently identified *A. xylosoxidans* correctly.

The CF community, the Centers for Disease Control and Prevention, and the American Society for Microbiology en-

TABLE 2. Activity of antimicrobial agents against *Alcaligenes* spp. isolated from CF patients

Antimicrobial agent tested	MIC (µg/ml) ^a			Susceptible isolates (%)
	Range	50%	90%	
Ticarcillin-clavulanic acid	4->128	128	>128	40
Piperacillin	4->128	32	>128	50
Piperacillin-tazobactam	4->128	32	>128	55
Ceftazidime	2->64	64	128	45
Imipenem	1->16	4	>16	59
Meropenem	0.5->16	8	>16	51
Ciprofloxacin	0.5->8	>8	>8	9
Tobramycin	4->256	>256	>256	3
Trimethoprim-sulfamethoxazole	>16	>16	>16	0
Chloramphenicol	8->64	32	>64	22
Minocycline	1-32	8	16	51
Colistin	100->200			92

TABLE 3. Activity of antimicrobial agents against *Alcaligenes* spp. isolated from CF patients

Antimicrobial combination	Concn tested (μg/ml) (range) ^a	% Synergistic activity	% Additive activity
Ciprofloxacin-piperacillin	0.5–4, 8–256	6	10
Ciprofloxacin-imipenem	0.5–4, 0.5–16	5	27
Ciprofloxacin-meropenem	0.5–4, 0.5–16	9	23
Ciprofloxacin-trimethoprim-sulfamethoxazole	0.5–16, 1–8	1	0
Ceftazidime-trimethoprim-sulfamethoxazole	1–32, 1–8	4	3
Ceftazidime-chloramphenicol	1–32, 4–32	2	14
Chloramphenicol-trimethoprim-sulfamethoxazole	4–32, 1–32	1	12
Chloramphenicol-minocycline	4–32, 1–32	5	35

^a The first and second ranges of values given are for the first and second parts of the antimicrobial combination, respectively.

dorse the use of selective media for processing respiratory tract specimens from patients with CF (11, 15, 28). However, there has not been a consensus for the use of selective media other than those for *B. cepacia* complex, e.g., OFPBL agar or *B. cepacia* selective medium (13). More extensive recommendations for the use of selective media, which include mannitol salt agar, chocolate agar, sheep blood agar, and MacConkey agar, have been made by experts in CF microbiology (23). Furthermore, site visits to accredited CF care centers sponsored by the Cystic Fibrosis Foundation are made approximately every 4 years by a member of the CF center directors committee (Preston Campbell, personal communication). During these site visits, the directors of the clinical microbiology laboratories are interviewed to assess their understanding of the importance of appropriate processing of respiratory tract specimens from CF patients, including the use of selective media, and the importance of understanding newly emerging pathogens. While clinical microbiology laboratories do have their own written procedures for processing CF specimens, the American Society for Microbiology should develop and publish national standards for processing CF specimens.

The most recent NCCLS documents do not include recommendations for the optimal antimicrobial susceptibility testing method for *Alcaligenes* spp. nor the antimicrobial agents that should be tested. We utilized a microbroth dilution assay that we have shown to be comparable with agar dilution for testing the antimicrobial susceptibility of *P. aeruginosa* isolates from CF patients (25). This method, as well as the disk diffusion method, has been endorsed by the NCCLS for susceptibility testing of *P. aeruginosa* isolates from CF patients (18). We tested a panel of antimicrobial agents with activity against *P. aeruginosa* (24) and *B. cepacia* (2) and used susceptibility breakpoints established for *P. aeruginosa*. Notably, minocycline, an agent not usually included in automated commercial microbroth dilution systems, was found to be active against 51% of the isolates of *A. xylosoxidans* tested. The carbapenem agents and piperacillin, with or without tazobactam, were also active against these isolates of *A. xylosoxidans*. Earlier studies have demonstrated that imipenem, ceftazidime, β -lactamase inhibitor combinations, and trimethoprim-sulfamethoxazole

were most active (12, 27), although these studies did not include CF isolates.

While the clinical efficacy of synergy studies has not yet been tested, combinations of antimicrobial agents are often recommended for treatment of highly resistant pathogens such as *P. aeruginosa* and *S. maltophilia*. Thus, our findings may have implications for treatment of CF patients as well as non-CF patients with nosocomial infections caused by *A. xylosoxidans* (5).

There are several limitations to this study. Patients with CF frequently harbor more than one pathogen, and the referred specimens may have been mislabeled. At present, the optimal method of antimicrobial susceptibility testing for *Alcaligenes* spp. is unknown, and there are limited reports assessing in vivo efficacy of antibiotics in either CF or non-CF patients. The optimal methodology for susceptibility testing of colistin is under investigation (9, 14), as is the concentration deliverable by aerosolization (30).

In conclusion, further studies are needed to standardize microbiologic methodologies for identification and susceptibility testing of *Alcaligenes* spp. in both CF and non-CF patients. In addition, future studies should continue to address the epidemiology and the possible pathogenic role of *A. xylosoxidans* in CF patients. A methodology, such as API 20 NE, should be employed to verify identification of this species.

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